POLYNUCLEAR AROMATIC HYDROCARBONS

METHOD 610

1. Scope and Application

1.1 This method covers the determination of certain polynuclear aromatic hydrocarbons (PAH). The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Benzo(a)anthracene	34526	56-55-3
Benzo(a)pyrene	34247	50-32-8
Benzo(b)fluoranthene	34230	205-99-2
Benzo(ghi)perylene	34521	191-24-2
Benzo(k)fluoranthene	34242	207-08-9
Chrysene	34320	218-01-9
Dibenzo(a,h)anthracene	34556	53-70-3
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Indeno(1,2,3-cd)pyrene	34403	193-39-5
Naphthalene	34696	91-20-3
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0

1.2 This is a chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for many of the parameters listed in Section 1.1, using the extract produced by this method.

1.3 This method provides for both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) approaches to the determination of PAHs. The gas chromatographic procedure does not adequately resolve the following four pairs of compounds:

anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)-anthracene and indeno(1,2,3-cd)pyrene. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the liquid chromatographic approach must be used for these compounds. The liquid chromatographic method does resolve all 16 of the PAHs listed.

1.4 The method detection limit (MDL, defined in Section 15)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ depending upon the nature of interferences in the sample matrix.

1.5 The sample extraction and concentration steps in this method are essentially the same as in Methods 606, 608, 609, 611 and 612. Therefore, a single sample may be extracted to measure the parameters included in the scope of each of these methods, provided the concentration is high enough to permit selecting aliquots of the extract for cleanup, when required. Selection of the aliquots must be made prior to the solvent exchange steps of this method. The analyst is allowed the latitude, under Gas Chromatography (Section 13), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.7 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and GC and in the interpretation of liquid and gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. The solvent is exchanged to cyclohexane prior to cleanup. Following cleanup, when using HPLC for determination of the PAHs, the solvent is exchanged to acetonitrile. Ultraviolet (UV) and fluorescence detectors are used with HPLC. When cleanup is not required and when FIDGC is used for determination, the methylene chloride extract may be analyzed directly. When cleanup is required, the cyclohexane exchange is made. Instrumental conditions are described which permit the separation and measurement of the PAH compounds[2].

2.2 A silica gel column cleanup procedure is provided to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional clean-up approaches to achieve the MDL listed in Table 1.

3.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets

should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[4-6] for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens; benzo(a)anthracene, benzo(a)pyrene and dibenzo(a,h)-anthracene.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes

K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity, with Teflon lined screw-cap.

5.2.8 Chromatographic column - 250 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 min. or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001g.

5.6 High performance liquid chromatographic apparatus (modular):

5.6.1 Gradient pumping system, constant flow.

5.6.2 Reverse phase column, 5 micron HC-ODS Sil-X, 250 mm x 2.6 mm ID (Perkin Elmer No. 089-0716 or equivalent).

5.6.3 Fluorescence detector, for excitation at 280 nm and emission greater than 389 nm cutoff (Corning 3-75 or equivalent). Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector.

5.6.4 UV detector, 254 nm, coupled to fluorescence detector.

5.6.5 Strip chart recorder compatible with detectors. Use of a data system for measuring peak areas and retention times is recommended.

5.7 Gas chromatograph - An analytical system complete with temperature programmable gas chromatograph suitable for on-column injection or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.7.1 Column - 1.8 m long x 2 mm ID pyrex glass packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 2. Guidelines for the use of alternate column packings are provided in Section 13.

5.7.2 Detector - Flame ionization. This detector has proven effective in the analysis of wastewaters for the compounds

listed in the scope excluding the four pairs of unresolved compounds listed in Section 1.3. Guidelines for the use of alternate detectors are provided in Section 12.2.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Sodium thiosulfate - (ACS) Granular.

6.3 Cyclohexane, methanol, acetone, methylene chloride, and pentane - Pesticide quality or equivalent.

6.4 Acetonitrile, high purity water-HPLC quality, distilled in glass.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for 4 hrs. in a shallow tray.

6.6 Silica gel - Grade 923 (100/200 mesh) dessicant (Davison Chemical or equivalent). Before use, activate for at least 16 hours at 130°C in a shallow glass tray, loosely covered with foil.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in HPLC quality acetonitrile, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, in Cincinnati, Ohio.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish liquid or gas chromatographic operating parameters to produce resolution of the parameters equivalent to that indicated in Tables 1 or 2. The chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Analyze each calibration standard (5 to 25 µL for HPLC and 2 to 5 µL for GC), and tabulate peak height or area responses against the mass injected. The results may be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at

a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Analyze each calibration standard (5 to 25 µL for HPLC and 2 to 5 µL for GC) and tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using Equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate

the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetronitrile 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 3, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U. S. EPA plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

$$\text{Upper Control Limit (UCL)} = R + 3s$$
$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[7].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 14.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be

combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles and protected from light.

10.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1 to 10 mL aliquot of sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a micro-Snyder column. Prewet the micro-Snyder column by

analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as chromatography with a dissimilar column or detector must be used. This may include the use of a mass spectrometer. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive, therefore, samples, extracts and standards should be stored in amber or foil wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottle and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine[9]. Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days, and analysis completed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle, rinse and repeat the extraction procedure a second time,

adding 0.5 mL methylene chloride to the top. Place the micro-K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 mL.

11.3 Silica gel column cleanup for PAHs.

11.3.1 Prepare a slurry of 10g activated silica gel in methylene chloride and place this in a 10-mm ID chromatography column. Gently tap the column to settle the silica gel and elute the methylene chloride. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 40 mL of pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL of cyclohexane sample extract onto the column, using an additional 2 mL of cyclohexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 25 mL pentane and continue elution of the column. Discard the pentane eluate.

11.3.4 Elute the column with 25 mL of methylene chloride/pentane (4 + 6)(V/V) and collect the eluate in a-500 mL K-D flask equipped with a 10-mL concentrator tube. Elution of the column should be at a rate of about 2 mL/min.

11.3.5 Concentrate the collected fraction to less than 10 mL by K-D techniques as in Section 10.6, using pentane to rinse the walls of the glassware. Proceed with HPLC or gas chromatographic analysis.

12. High Performance Liquid Chromatography (HPLC)

12.1 To the extract in the concentrator tube, add 4 mL of acetonitrile and a new boiling chip, then attach a micro-Snyder column. Increase the temperature of the hot water bath to 95 to 100°C. Concentrate the solvent as in Section 10. After cooling, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust the extract volume to 1.0 mL.

12.2 Table 1 summarizes the recommended HPLC column materials and

operating conditions for the instrument. This Table includes retention times, capacity factors, and method detection limits that were obtained under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenapthene, and fluorene and the fluorescence detector is recommended for the remaining PAHs. Examples of the parameter separations achieved by this HPLC column are shown in Figures 1 and 2. Other HPLC columns, chromatographic conditions or detectors may be used if the requirements of Section 8.2 are met.

12.3 Calibrate the system daily as described in Section 7.

12.4 If the internal standard approach is being used, the internal standard must be added to sample extract and mixed thoroughly, immediately, before injection into the instrument.

12.5 Inject 5 to 25 µL of the sample extract using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 µL, and the resulting peak size in height or area units. Re-equilibrate the LC column at the initial gradient conditions for at least 10 minutes between injections.

12.6 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.7 If the peak height or area exceeds the linear range of the system, dilute the extract with acetonitrile and reanalyze.

12.8 If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

13. Gas Chromatography

13.1 The packed column gas chromatographic procedure will not resolve certain isomeric pairs as indicated in Section 1.3 and Table 2. The liquid chromatographic procedure (Section 12) must be used for these materials. Capillary (open-tubular) columns may be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.2 To achieve maximum sensitivity with this method, the extract must be concentrated to 1.0 mL. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methylene chloride to the top. Place the micro K-D

apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus. Drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

13.3 Table 2 describes the recommended gas chromatographic column and operating conditions for the instrument. This Table includes retention times that were obtained under these conditions. An example of the parameter separations achieved by this column is shown in Figure 3. Other packed columns, chromatograhic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.4 Calibrate the gas chromatographic system daily as described in Section 7.

13.5 If the internal standard approach is being used, add the internal standard to sample extract and mix thoroughly, immediately, before injection into the instrument.

13.6 Inject 2 to 5 µL of the sample extract using the solvent-flush technique[10]. Smaller (1.0 µL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

13.7 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13.8 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

13.9 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. Calculations

14.1 Determine the concentration of individual parameters in the sample.

14.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from Equation 2:

$$\text{Eq. 2.} \quad \text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (µL).
V_t = Volume of total extract (µL).
V_s = Volume of water extracted (mL).

14.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

$$\text{Eq. 3.} \quad \text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Amount of internal standard added to each extract (µg).
V_o = Volume of water extracted, in liters.

14.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

14.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits established in Section 8.4, data for the affected parameters must be labeled as suspect.

15. Method Performance

15.1 Method detection limits - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentrations listed in Table 1 were obtained using reagent water[11]. Similar results were achieved using representative wastewaters. MDL for the GC approach were not determined.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 8 x MDL to 800 x MDL[11], with the following exception: benzo(ghi)perylene recovery at 80 x and 800 x MDL were low (35% and 45% respectively).

15.3 In a single laboratory (Battelle Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 3 were obtained[2]. Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the precent recovery is also included in Table 3.

15.4 The Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. Appendix A.

2. "Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters," Report for EPA Contract 68-03-2624 (In preparation).

3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

4. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

8. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

9. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

10. Burke, J. A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," Journal of the Association of Official Analytical Chemists, 48, 1037 (1965).

11. Cole, T., Riggins, R., and Glaser, J., "Evaluation of Method Detection Limits and Analytical Curve for EPA Method 610 - PNAs," International Symposium on Polynuclear Aromatic Hydrocarbons, 5th, Battelle Columbus Laboratory, Columbus, Ohio (1980).

TABLE 1

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min)	Capacity Factor (k')	Method Detection Limit (µg/L)[a]
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.64
Anthracene	23.4	17.6	0.66
Fluoranthene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)fluoranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 mm x 250 mm Perkin-Elmer column; isocratic elution for 5 min. using acetonitrile/water (4 + 6), then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate is 0.5 mL/min. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

[a]The method detection limit for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2

GAS CHROMATOGRAPHIC OPERATING CONDITIONS
AND RETENTION TIMES

Parameter	Retention Time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

GC conditions: Chromosorb W-AW-DCMS (100/120 mesh) coated with 3% OV-17, packed in a 6' x 2 mm ID glass column, with nitrogen carrier gas at a flow rate of 40 mL/min. Column temperature was held at 100°C for 4 minutes, then programmed at 8°/minute to a final hold at 280°C.

TABLE 3

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
Acenaphthene	88	5.7	11.6-25	24	4
Acenaphthylene	93	6.4	250-450	24	4
Anthracene	93	6.3	7.9-11.3	24	4
Benzo(a)anthracene	89	6.9	0.64-0.66	24	4
Benzo(a)pyrene	94	7.4	0.21-0.30	24	4
Benzo(b)fluoranthene	97	12.9	0.24-0.30	24	4
Benzo(ghi)perylene	86	7.3	0.42-3.4	24	4
Benzo(k)fluoranthene	94	9.5	0.14-6.2	24	4
Chrysene	88	9.0	2.0-6.8	24	4
Dibenzo(a,h)anthracene	87	5.8	0.4-1.7	24	4
Fluoranthene	116.	9.7	0.3-2.2	24	4
Fluorene	90.	7.9	6.1-23	24	4
Indeno(1,2,3-cd)pyrene	94	6.4	0.96-1.4	24	4
Naphthalene	78	8.3	20-70	24	4
Phenanthrene	98	8.4	3.8-5.0	24	4
Pyrene	96	8.5	2.3-6.9	24	4

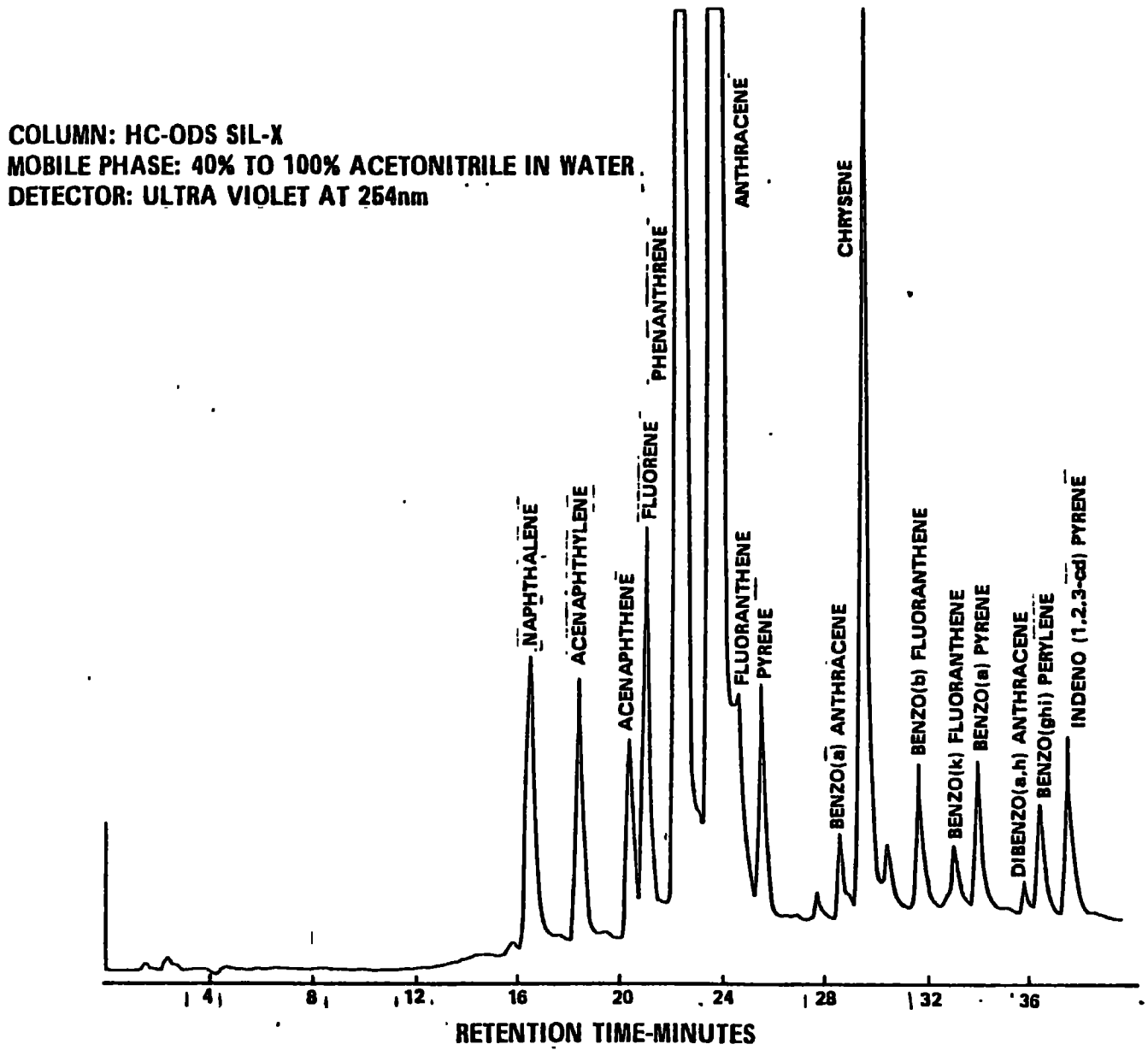

Figure 1. Liquid chromatogram of polynuclear aromatic hydrocarbons.

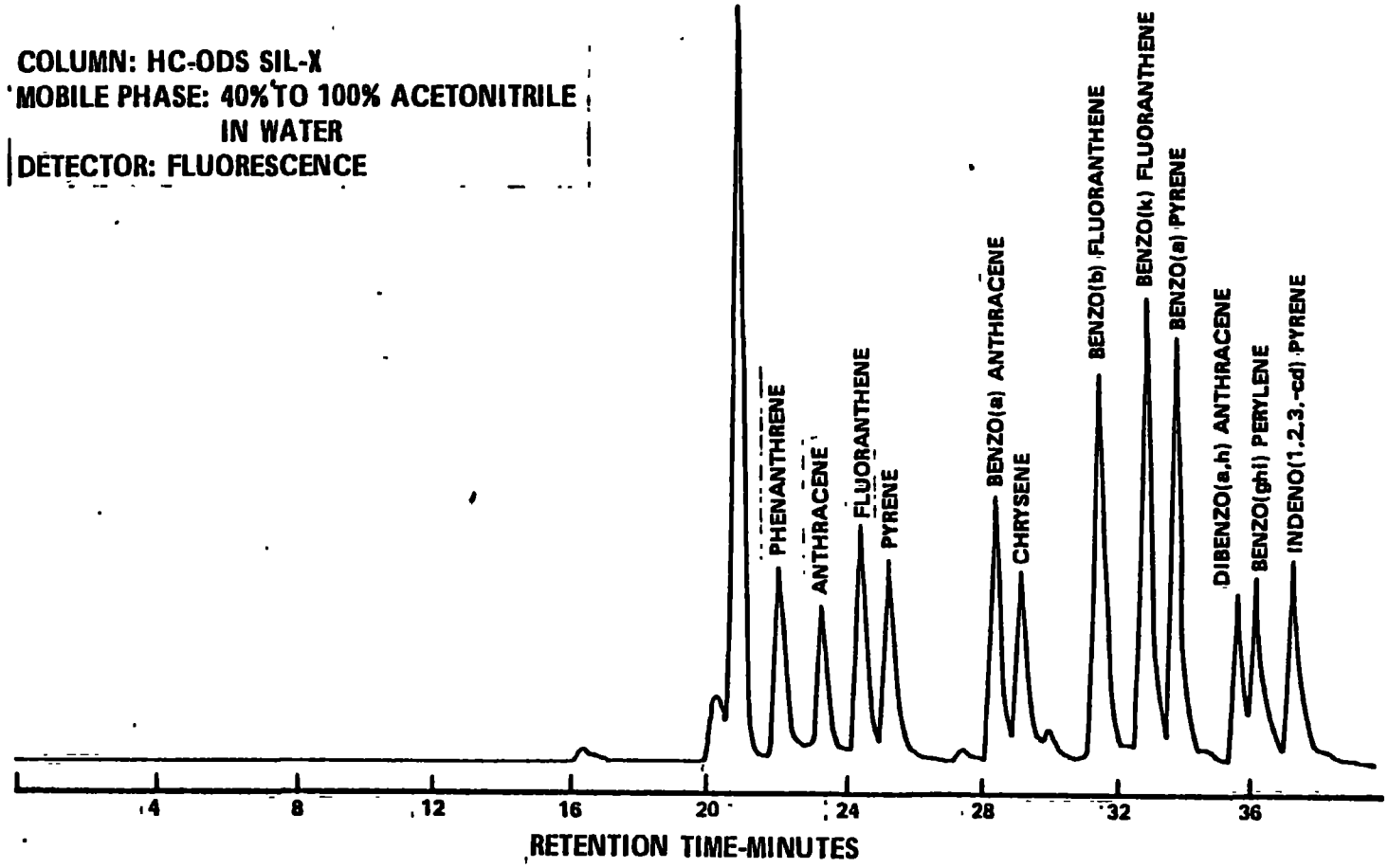

Figure 2. Liquid chromatogram of polynuclear aromatic hydrocarbons.

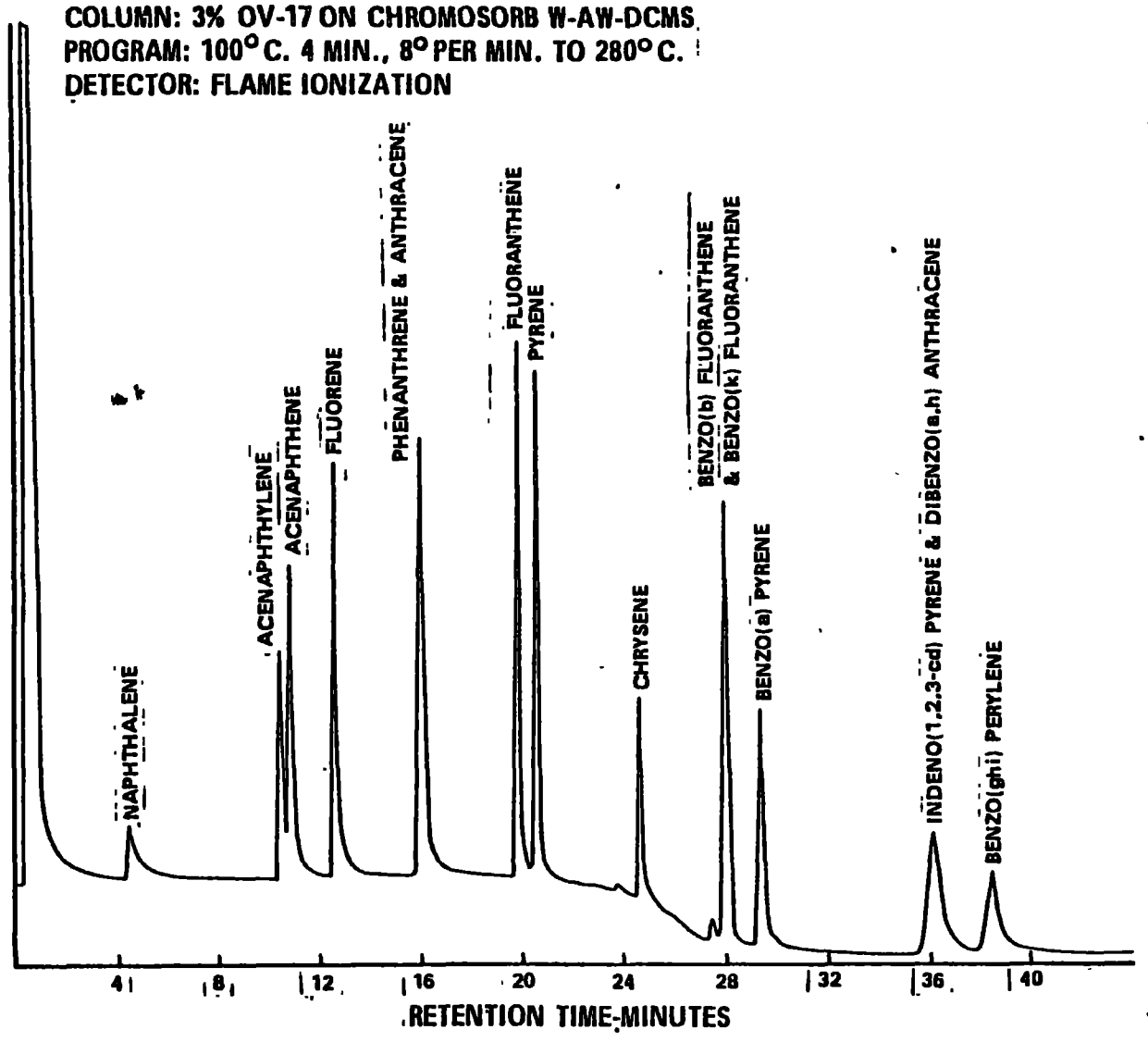

Figure 3. Gas chromatogram of polynuclear aromatic hydrocarbons.